A Framework for a Computational Toxicology Research Program in ORD

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A FRAMEWORK FOR A COMPUTATIONAL TOXICOLOGY RESEARCH PROGRAM IN ORD

OFFICE of RESEARCH and DEVELOPMENT
US ENVIRONMENTAL PROTECTION AGENCY

Disclaimer

This document does not constitute an Agency position or policy concerning computational toxicology. Any mention of trade names does not constitute Agency endorsement. This document has been reviewed internally for clearance. Additional revisions in this document will occur following a consultation with the Agency's Science Advisory Board and comments from a Workshop on Computational Toxicology. Both of these meetings are scheduled for September, 2003. The revised document will be subject to external peer review.

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LIST OF FIGURES

ACRONYMS

AR- androgen receptor
BBDR Models-Biologically Based, Dose-Response Models
CAFO-Concentrated Animal Feeding Operation
CEBS-Chemical Effects in Biological System
CNS- Central Nervous System
DBPs-Disinfectant By-Products
DOE-Department of Energy
EDCs-Endocrine Disrupting Chemicals
EDSTAC-Endocrine Disruptor Screening and Testing Advisory Committee
EMAP-Environmental Monitoring and Assessment Program
FQPA-Food Quality Protection Act
HPG/T Axis-Hypothalamic-Pituitary-Gonadal/Thyroid Axis
HTPS-High Through-Put Screening
JGI-Joint Genome Institute
LC-Lethal Concentration
LD-Lethal Dose
MOA-Mode or Mechanism of Action
MOU-Memo of Understanding
NCEA-National Center for Environmental Assessment
NCER- National Center for Environmental Research
NCT-National Center for Toxicogenomics
NERL- National Exposure Research Laboratory
NHEERL- National Health and Environmental Effects Research Laboratory
NIEHS-National Institute of Environmental Health Sciences
NMR-Nuclear Magnetic Resonance
NRMRL- National Risk Management Research Laboratory
ORD-Office of Research and Development
PBPK Models-Physiologically Based, Pharmacokinetic Models
PC- Personal Computer
PD- Pharmacodynamic
PFOS-Perfluorooctane Sulfonate
PK- Pharmacokinetic
QSAR-Quantitative Structure Activity Relationships
RFA-Request for Application
SAB-Science Advisory Board
SAR-Structure Activity Relationship

EXECUTIVE SUMMARY

The mission of the U. S. Environmental Protection Agency is to safeguard public health and the environment from adverse effects that may be caused by exposure to pollutants in the air, water, soil and food. Protecting human health and the environment carries with it the challenge of assessing possible hazardous effects for tens of thousands of chemicals. The large number of chemicals that the Agency must consider under many different regulations together, with the large cost of conducting test batteries, limits the full use of standard toxicity test methods to only a small number of chemicals. The Agency is also faced with reducing uncertainties associated with performing quantitative risk assessments on chemicals for which data have been submitted by the chemical industries.

Over the last several years, there has been increased pressure to utilize novel technologies derived from computational chemistry, molecular biology and systems biology in toxicological risk assessment. This new area has been referred to as "Computational Toxicology", which is defined in this document as the application of mathematical and computer models for prediction of effect and the understanding of mechanism of action. This document describes a framework for the development of a research program within the Agency's Office of Research and Development (ORD) to utilize approaches derived from modern computational methods, molecular biology, and systems biology to address the questions of "when and how" to test specific chemicals for hazard identification and to improve quantitative dose-response assessment.

In assessing risk associated with exposure to a chemical or other environmental stressor, there are a number of uncertainties that lie along a continuum, including the presence of the chemical in the environment, the uptake and distribution of the chemical in the organism or human or environment, the presence of the active chemical at a systemic target site, and the series of biological events that lead to the manifestation of an adverse outcome. The overall goal of ORD's research program on Computational Toxicology is to use emerging technologies to improve quantitative risk assessment by reducing uncertainties in this source-to-adverse outcome continuum. The three strategic objectives of the Computational Toxicology Program are to develop: 1) improved linkages across the source-to-outcome continuum, 2) approaches for prioritizing chemicals for subsequent screening and testing, and 3) better methods and predictive models for quantitative risk assessment. With regard to improving linkages between source-to-outcome, how computational toxicological approaches could be used is discussed for a number of links along the exposure-to-effect continuum, including chemical transformation and metabolism, better diagnostic/prognostic molecular markers, improved dose metrics, characterization of toxicity pathways, metabonomics, system biological approaches, and modeling frameworks and uncertainty analysis. Computational toxicological approaches are also needed to develop better predictive models for screening and testing, including quantitative structure activity relationship (QSAR) models, improved pollution prevention strategies, and approaches to high through-put screening. Computational toxicological approaches will also be used to address a number of research needs associated with dose-response assessment, cross-species extrapolation, and

assessment of the effects of chemical mixtures.

The research program at ORD currently uses many computational and biological approaches that fall under the general area of computational toxicology, and examples of such work are described in this document. Other research agencies such as the National Institute of Environmental Health Sciences and the Department of Energy have significantly greater capabilities for research on computational toxicology than the Agency. ORD has initiated discussions with these agencies in order to facilitate the development of a national approach to the use of computational procedures in toxicology.

This document is intended to identify the research needs and unique capabilities of ORD laboratories to provide the basis for a more focused and integrated research program in the future. To accomplish this, ORD proposes consultation on this framework by the Agency's Science Advisory Board (SAB) followed by a workshop with scientists from other research organizations having complementary research capabilities. Based on comments from the SAB and the workshop, ORD will develop an implementation plan to guide research on computational toxicology over the 5-10 years.

I. INTRODUCTION

A. The Computational Toxicology Program

> **Computational Toxicology** is the application of mathematical and computer models for prediction of effect and the understanding of mechanism.

The overall objective of this document is to describe a framework for the development of a research program in Computational Toxicology by the Environmental Protection Agency's (the Agency) Office of Research and Development (ORD). Computational toxicology involves the application of various mathematical and computer models to predict effects and understand the cascade of events that result in an adverse response, or its mode or mechanism of action.

The Computational Toxicology Research Program is a technology-based, hypothesis-driven effort to increase the soundness of risk assessment decisions within the Agency. It is designed to increase the capacity to prioritize, screen, and evaluate chemicals by enhancing the predictive understanding of toxicities. Success will be measured by the ability to improve risk assessments by understanding the potential of chemicals to affect molecular and biochemical pathways of concern, i.e., their toxicity pathways.

> **Computational Toxicology Involves:**
>
> Computational chemistry, which refers to the physical-chemical mathematical modeling at the molecular level and includes such topics as quantum chemistry, force fields, molecular mechanics, molecular simulations, molecular modeling, molecular design, and cheminformatics;
>
> Computational biology or bioinformatics, which involves the development of molecular biology databases and the analysis of the data; and
>
> Systems biology, which refers to the application of mathematical modeling and reasoning to the understanding of biological systems and the explanation of biological phenomena.

In the area of computational biology, recent advances in "omic" technology make this a particularly appropriate time for such a program. Current research in this area focuses on sequencing whole genomes and understanding the complexity of cellular biology at the molecular level. The development of "omic" technologies has evolved into three scientific disciplines: genomics which is defined as the study of genes and their function, proteomics which is defined as the study of the full set of proteins encoded by a genome, and metabonomics, which is defined as the study of the total metabolite pool. Several recent technological advances now make it possible to develop molecular profiles using genomic, proteomic, and metabonomic methods in

order to identify the impacts that chemicals have on living organisms or the environment. Although the technology continues to change and improve, conducting these types of analyses is no longer a question of capability. The use of these "omic" technologies to study toxicological questions is called toxicogenomics. Traditionally, the EPA has used the terms "mode of action" to refer to the key events and processes that lead to an adverse outcome, and "mechanism of action" to refer to a more detailed understanding and description of events than is meant by mode of action. Because the genomic technologies available today allow for the molecular profiling at multiple levels of biological organization with a breadth that has not been possible in the past, this document uses the term "toxicity pathway" to denote that enhancement.

Parallel to efforts in computational biology, there have been major advances in computational speed and access to data. Less than a decade ago, describing the complexity of chemical behavior in biological systems was severely limited because realistic models presented combinatorial and other problems beyond the capabilities of most computers. In the field of bioinformatics, for example, major advances were made not from faster statistical analysis of data after its acquisition, but from the integration of computational and data acquisition technologies. It is now possible to consider how to evaluate the vast amounts of information generated by "omic" technologies using data mining tools made possible by rapid advances in computational storage capacity and speed.

One area where computational toxicology has shown promise is in the discipline of physical organic chemistry known as Quantitative Structure Activity Relationships (QSAR). Application of QSAR has resulted in developing novel predictive capabilities for representing chemical structures as a distribution of conformations and properties rather than discrete structures. Another promising area brought about by the joining of computer science, biology and medical programs is an emerging discipline known as systems biology which could lead to the development of virtual biological systems.

B. Application of Computational Toxicology to Risk Assessment and Research

ORD's research programs support the Agency's regulatory decision-making by providing scientific information for human health and ecological risk assessment. Risk assessment is the process used to evaluate the hazards of and exposures to environmental stressors to produce estimates of the probability that populations or individuals will be harmed by chemical exposure and to what degree. It is one component of the process by which the Agency, and many other organizations, recognize a potential risk and decide how to respond.

The Agency's risk assessments of chemicals rely primarily on laboratory testing on a chemical-by-chemical basis to obtain data about adverse effects and the quantitative relationship between dose level and likelihood of response. In human health risk assessment, these laboratory

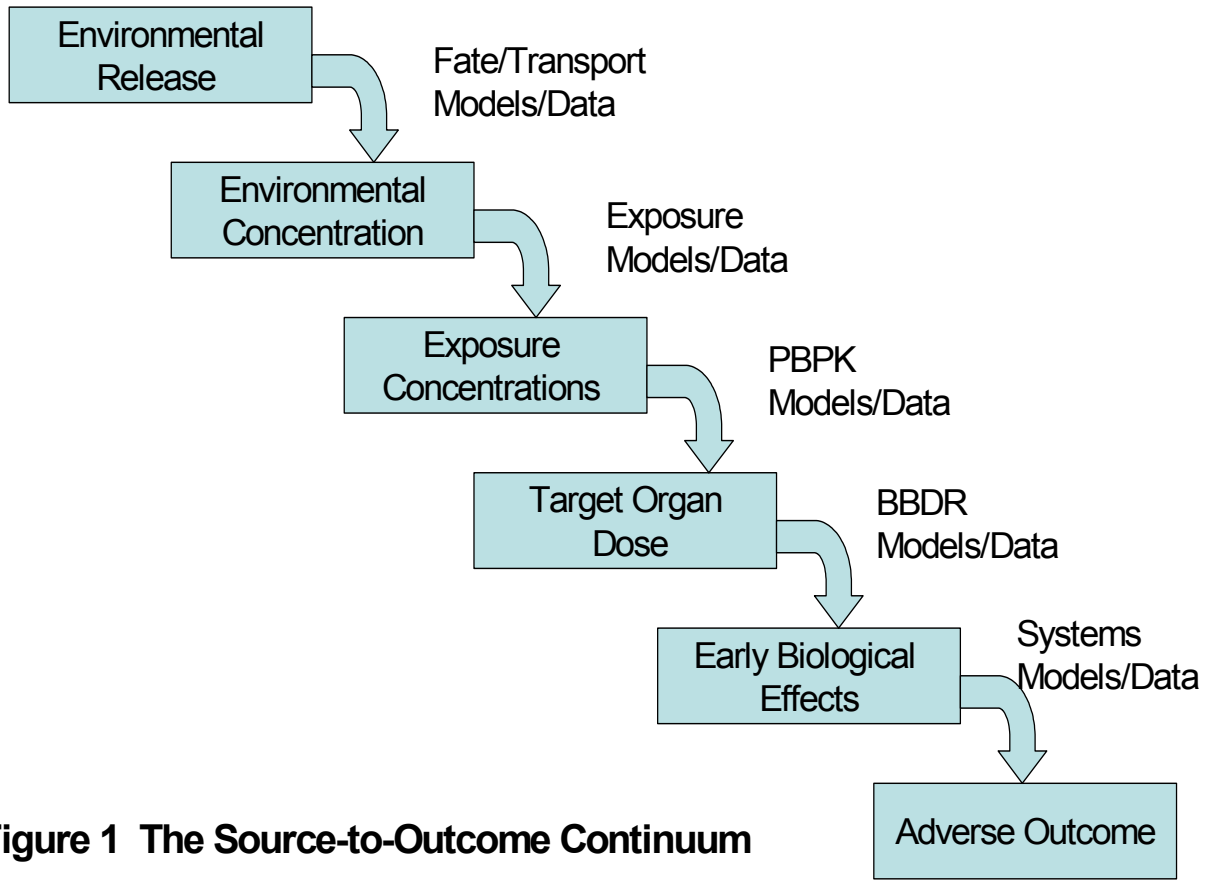

Figure 1 The Source-to-Outcome Continuum

data are extrapolated to humans to estimate human risk. The large number of chemicals in commerce coupled with the expense of laboratory testing limits the application of extensive standard toxicity testing to relatively few chemicals. ORD will also explore the feasibility of using computational approaches to improve quantitative dose-response assessment and the development of sensitive and specific tests for hazard identification. Preliminary efforts in this area began in FY02 with a Congressional reprogramming action to explore the use of alternatives to animal testing for hazard identification. ORD interpreted this as an opportunity to evaluate genomic and computational tools for screening purposes and it initiated several research projects on endocrine disruptors as a 'proof-of-concept' effort. Endocrine disruptors were selected because it was felt that a considerable amount of knowledge on mechanisms of actions and toxicity pathways existed for this class of environmental pollutants. This feasibility effort is described in greater detail in III.A.

C. Overall Goal and Strategic Objectives

ORD has found it useful to envision the risk assessment paradigm as a continuum of events leading from release in the environment to adverse effect. Figure 1 is a simplification of this concept showing points along the continuum where a measurement or an observation can be made. The arrows between the boxes represent a cascade of events that lead from one measurable

event to the next. ORD's research program focuses on learning more about the processes that lead from exposure to adverse outcome because this will allow the Agency to perform better risk assessments.

Objectives of the Computational Toxicology Program

Improve Linkages in Source-to-Outcome Paradigm
Provide Predictive Models for Hazard Identification
Enhance Quantitative Risk Assessment

The overall goal of ORD's research program on Computational Toxicology is to use the tools of modern chemistry, biology and computing to provide the Agency with the tools to improve quantitative risk assessments and reduce uncertainties in the source-to-outcome continuum. To meet this goal, ORD has identified three strategic objectives for the Computational Toxicology Research Program. First, research is needed to develop improved linkages across the source-to-outcome paradigm. Understanding those linkages will decrease uncertainties in assessing risk to human health and the environment. Second, research is needed to develop strategies for prioritizing chemicals for subsequent screening and testing. The current approach for screening and testing chemicals requires extensive resources. Therefore, an approach must be developed to determine which chemicals or classes of chemicals in the universe of chemicals should be screened and tested first. Finally, research is needed to develop better methods and predictive models for quantitative risk assessment because current approaches take too long and are too costly. The following sections of this framework describe how ORD is currently using and how it proposes to use emerging technologies associated with computational toxicology to address the Agency's needs for developing approaches for screening and testing and for improving quantitative risk assessment.

II. RESEARCH NEEDS AND APPLICATIONS OF COMPUTATIONAL TOXICOLOGY TO GOALS

A. Improve Linkages in the Source-to-Outcome Paradigm

1. Chemical Transformation and Metabolism

At several points along the source-to-outcome continuum, it is critical to accurately model the fate of chemical stressors to determine the level of exposure to an organism. It is also crucial to accurately model the metabolism of a chemical inside the target organism, because it is often a metabolite of the original stressor that induces a biological event. In many cases, the reaction processes controlling the fate of chemicals outside of the organism are similar if not identical to those reaction processes controlling metabolism within the organism. For example, enzyme-mediated processes such as redox reactions and hydrolysis that result in the formation of

reactive intermediates (i.e., radicals, carbenes) that react irreversibly with biological receptors (e.g., DNA) are often the rate-determining processes controlling the fate of these chemicals in natural aquatic ecosystems. Consequently, the process of developing and refining simulators for environmental transformation and metabolism will have many commonalities.

a. Chemical Transformation in Ecosystems

The state of chemical fate and transport modeling for exposure assessment has advanced significantly in recent years. For example, it is now possible to forecast many of the physicochemical properties that ultimately govern chemical transformation. Nonetheless, many unknowns and uncertainties remain, and ORD continues to conduct research aimed at reducing them. There are several key areas of uncertainty, however, that can be reduced greatly by informing and validating fate models with molecular indicators of exposure. The rapid advances in "exposure genomics" (see Section II.A.2) will provide early signs of chemical exposure based on changes in gene expression, which will lead to the development of a new array of molecular indicators that can guide chemical fate and metabolism studies. The integration of genomics and molecular indicators into chemical fate studies can serve to improve linkages in the source-to-outcome continuum.

Chemical Fate Models

Determine minimal concentrations at which biological events occur
Determine biologically relevant chemical in mixtures
Focus studies on crucial biotransformation

The application of molecular indicators to chemical fate studies is several-fold. For chemicals that trigger biological events of concern, molecular indicators can be used to determine the minimal concentration at which the biological events occur. This approach will narrow the task of the exposure models to answering only the question of whether the toxicant (i.e., parent or reaction product) is above or below this minimal concentration, i.e., it will "bound" the model. Narrowing the model requirements can significantly increase certainty in the risk assessment process. Another area to address is the elucidation of the biologically relevant components in chemical mixtures by measuring changes in gene expression in exposed organisms. This application of molecular indicators can focus exposure models on a much smaller subset of candidate chemicals, including those potentially linked to initiation of adverse effects. Finally, molecular exposure indicators can be used to advance our understanding and ability to model biotransformations of chemicals in ecosystems. Biotransformation is widely recognized as the largest uncertainty in exposure modeling. Accurate predictive models for biotransformation have eluded scientists because the universe of enzyme reactions is so large. Gene expression tools will be used to narrow down this universe only to those that are biologically relevant, which will

enable more accurate and meaningful prediction.

b. Chemical Metabolism

Many toxic effects result from metabolic activation of parent chemicals to form metabolites that are much more toxic than the parent, and, thus, dominate the toxicology. Morever, many cross-species differences in toxic effects are the result of differences in detoxification. Consequently, an accurate computerized simulator of metabolism in the liver and other target tissues (e.g., kidney) is essential to meet the objectives of the program. The primary goal of this research is the development of a computational system that will predict and prioritize metabolic pathways for liver metabolism.

Metabolic Simulator

Libraries of relevant metabolic transformation
High quality data metabolic maps
Probability indices for substructural units

The first step in the development of a metabolic simulator is to create a library of all known metabolic transformations which are nested according to the substructural elements being transformed. Algorithms are then used to recognize the relevant substructural units in a chemical of concern that can undergo metabolism. The transformation products from each possible reaction are stored as a list of first-level metabolites. Each metabolite, in turn, is subsequently submitted to the substructural matching routine to generate a set of second-level metabolites from each first-level metabolite. The process is continued until the metabolic map is completed.

This approach to simulating metabolism tends to identify many metabolic candidates that are ultimately improbable because of kinetic considerations. This problem can be overcome by associating a probability with each substructural transformation process in the library. Such transformation probabilities can be derived statistically from a library of high quality metabolic maps. Unfortunately, currently available metabolism libraries have significant gaps in relative rates for many important metabolic reactions. Identification of these gaps will direct the generation of new high-quality data on metabolism. These data will be generated using traditional experimental methods and new advanced analytical techniques [e.g., wide-bore, high-resolution nuclear magnetic resonance (NMR)] for measuring metabolic rate constants and identifying metabolites *in vivo* and *in vitro*. Mechanistic-based predictive methods can also be used to fill some important data gaps.

2. Development of Diagnostic/Prognostic Molecular Indicators

Exposure risk assessment has historically been based on the use of chemical analysis data to generate exposure models. While the biological activity of chemicals has been recognized to be important for exposure risk assessments, the measurement of such activity has largely been limited to whole organism toxicity tests. Considerable less study has been done using *in vitro* tests to assess specific types of biological activity present in whole or fractionated environmental samples. Current *in vitro* capabilities are not sufficiently validated to address exposure risk assessment.

There is a need to develop cellular and molecular indicators of exposure that can be used to assess the vulnerability of humans and wildlife to single and multiple pathways of exposure to chemicals in the environment. However, correlation of such indicators will require a greater understanding of the linkage of the cellular or molecular indicator with specific cellular and tissue-level effects (e.g., reproductive or neurologic toxicity) or outcomes (e.g., fertility or neurological disease).

Diagnostic Indicator Studies

Few environmental stressors have specific or sensitive indicators
Exposure indicators are poorly correlated with effects
Molecular indicators could validate fate and transformation models
Crucial for mixtures risk assessment
Essential for integrated approach to risk assessment

The indeterminate condition of exposure indicator research stands to change remarkably with attempts to link molecular biological technologies with cellular or tissue effects and outcomes. The computational toxicology program aims to develop a platform or sequence of approaches where "the earliest recognizable signatures of exposure" (i.e., unique patterns of up- and down-regulation of genes) are identified for scores of different stressors, become user-friendly procedures, are demonstrated in case studies and then incorporated into the Agency, State and Regional studies supported by the Agency's Environmental Monitoring and Assessment Program (EMAP). Acting on the tenet that any response to or effect from a stressor will involve changes in the expression of some genes, gene discovery and DNA microarray synthesis and use are hypothesized to provide a window on hundreds of changes which may or may not be linked to downstream cascades of activity which lead to adverse effects. Bioinformatic tools will be used to discriminate unique signatures and families of signatures indicative of stressors or groups of stressors. The scope of this computational toxicology program component is moving past the use of few genes in an organism such as the ecotoxicology model fathead minnow (*Pimephales promelas*) to the use of hundreds of genes and gene homologues acquired by less direct alternate molecular methods. Ultimately, the scope of the approach will move to the level of 25,000 to 30,000 genes associated with the entire genome of selected organisms. The existence of hundreds of signal transduction pathways in cells of higher organisms, discovered through traditional

biochemistry, heighten the likelihood of unique exposure signatures for a great number of individual chemical stressors and families of stressors. Studies will be both adverse effect driven (initially, development of molecular indicators for early molecular events in sex steroid mediated mechanisms of toxicity) and empirically-based (clusters of activity identified from watershed or regional stream surveys). The development of " molecular diagnostic indicators of exposure" presents the opportunity for the simultaneous, "near real-time" measurement of biologically relevant exposures of organisms to multiple stressors in mixtures. Nanotechnological instrumentation and robotics offer the promise of extremely high throughput analysis of indicators that can allow for larger scale exposure studies at the watershed and regional levels to be undertaken.

In addition to chemicals, humans and wildlife are exposed to other environmental stressors such as microorganisms. Genomics technologies offer unique opportunities to discriminate changes that occur within cells that define a particular microorganism's pathogenicity. Exposure to microorganisms is also likely to be a factor in characterizing exposure of humans and wildlife to other stressors. Prediction of the outcome of chemical exposures stands to be significantly enhanced by an understanding of changes in cellular responses contributed by both biotic and abiotic influences. This information will be important for the development of criteria for drinking water.

There is a significant potential for integration of the use of molecular information used to address issues related to exposure risk assessment with other genomics and computational chemistry research. Research on chemical transformation in the environment and within organisms (dosimetry) will be more effective because of advances in computational chemistry. The application of genomics can also serve to validate the real life relevance of the transformation and physiologically based pharmacokinetic (PBPK) modeling of chemicals by integrating across parent chemicals and metabolites to reveal the "first recognizable signature of exposure" (changes in gene expression). Once discriminated, these molecular events will ultimately be linked to toxicity pathways as described in Section II.A.4.

3. Dose Metrics

Qualitative and quantitative evaluations of the relationship between dose and response are key components of the quantitative risk assessment process and could be improved with the use of computational methods. The choice of the chemical species and even the

Dose Metrics

Dose is often inferred from stressor uptake
Dose models stand to be enhanced with specific data on stressor interactions with molecules initiating toxicity pathways
Genetic polymorphism data will reduce uncertainty stemming from assumptions of homogeneous populations.
Susceptibility indicators will be developed for input into exposure models

actual dose metric for the risk assessment process depends upon the particular mode or modes of action being assessed. As the biological steps between the external exposure and some internal toxicologically relevant dose are often non-linear, pharmacokinetic (PK) models are often applied to link an exposure of interest to an observed adverse outcome. PBPK models that depend on knowledge of anatomy, physiology and biochemistry are the most common and typical examples of such models. In order for PBPK models to be used, several pieces of key information are needed. Some of this information, such as body size, organ volumes and blood flows are known for several species, including the human. Other important pieces of information, such as metabolic transformation rates, are chemical specific and may vary from species to species. The expense and time required to gain this information in laboratory studies has limited the use of this modeling technology. Another limitation to their use is the often lack of detailed knowledge on the molecular events that lead to toxicity, i.e., the toxicity pathway. The Computational Toxicology Research Program should enable broader use of PBPK by overcoming these limitations by providing better indicators of the relevant doses and receptors within the target organism.

Advancements made from this program in genomics and proteomics will better define the indicator of the most relevant doses for environmental chemicals entering the body. For example, specific binding to a particular part of the DNA, RNA, receptors, or enzymes might be a much more relevant dose metric than just the amount of chemical in a particular tissue. More specifically related biomarkers in easily obtained and measured biological fluids might also be developed from advances in computational toxicology which will be accurate indicators of some event inside key target cells.

It is also expected that developments in the science of genomics will greatly help in defining and characterizing sensitive sub-populations. It has long been recognized that not all members of the population are equally sensitive to the same environmental pollutant. While all the factors are not understood, advances in genomics and clinical medicine are showing that for many adverse processes some key component on the genome is impacted. That in turn then increases that individual's vulnerability to specific clinical disease. The field of metabonomics likewise offers new opportunities in characterizing variation in metabolic processes at the cellular and tissues levels. Integration of studies of stressor dose, transformation and metabolic sequelae have the potential to reveal the clearest perspective yet on relevant dose and its variation across organisms and populations. Subsequently, the contribution of genetic predisposition can be studied.

Advances in molecular biological science allow for the characterization of the genetic variation (polymorphisms) within populations. In turn, genetic marker data are defining the contribution of genetic variation to the overall level of variation in dose-response in organisms. These data are providing correlations between genotypes in the population and phenotypes such as altered transformation of stressors. Ultimately, integrated research in genetics and genomics has

the potential to elucidate specific altered molecular processes associated with genotypes representative of sensitive or vulnerable subpopulations. Dosimetry models such as PK and pharmacodynamic (PD) models can then incorporate these data to reduce the uncertainties associated with assuming populations are homogeneous regarding toxic response to stressors. Indicators in body fluids of individuals identified in susceptible subpopulations may also be developed to provide simple methods for the inclusion of relevant population data to exposure characterization within human and ecological risk assessments.

In summary, technologies developed as a result of this program will help in better defining toxicologically relevant doses. Techniques from this program will also help provide necessary information to develop good mechanistically based quantitative models for estimating the relevant doses and assessing their impact on the organisms affected.

4. Characterization of Toxicity Pathways

Understanding Toxicity Pathways

Identification of discrete molecular initiating events
Linking adverse outcomes to molecular alterations
Elucidating linkages across biological levels of organization
Biological basis for cross-species extrapolation
Prediction of possible interactions for untested chemicals and mixtures

Computational toxicology techniques have excellent promise to focus research on reducing uncertainties in both ecological and human health risk assessments. However, use of key predictive toxicology tools/approaches, including PBPK and QSAR models and/or alterations in gene (or protein) expression profiles, is useful only in the context of a thorough understanding of toxicity pathways of concern (i.e., the mechanism or mode of action). Specifically, for these types of predictive methods to be truly useful, it is necessary to link adverse outcomes (e.g., reproductive or developmental changes, cancer) to initiating events, ideally through the cascade of biochemical and physiological changes that occur as a result of the initial interaction(s) of xenobiotics with biological molecules (e.g., receptor binding, enzyme inhibition). A particularly key aspect of this is identification of the proximal (often initial) biological alteration associated with any particular toxicity pathway. For example, chemicals which bind to and activate specific nuclear receptors elicit a relatively predictable suite of biochemical and physiological responses that are species/class-specific, but culminate in very similar adverse reproductive and developmental effects across numerous vertebrate species. Identification of common initiating events, such as receptor activation, can enable the successful use of models or gene expression assays to deal with xenobiotics as classes of compounds rather than individual chemicals. Further, through understanding the cascade of events that occur as a result of receptor activation, in conjunction with accurate dosimetry predictions, it would become possible to predict adverse outcomes associated with exposure to, as yet, untested chemicals. For this to be feasible, an understanding of toxicity pathways based on discrete initiating events is needed.

Linking Observations Across Levels of Biological Organization

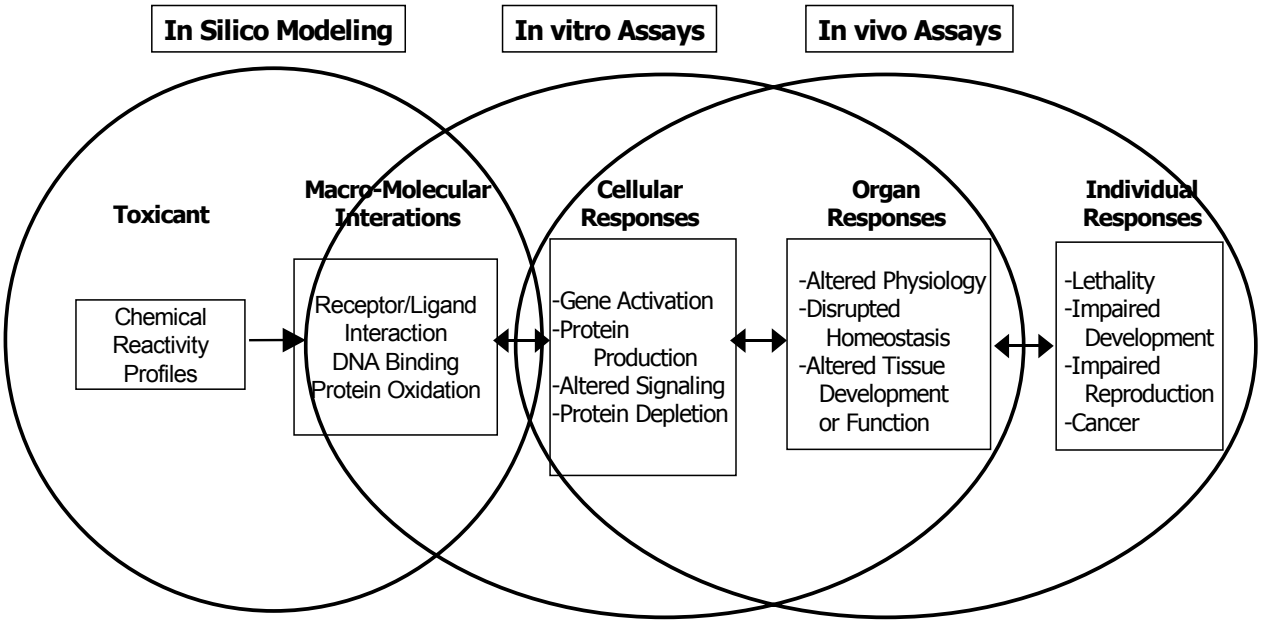

Figure 2 An Example of a Toxicity Pathway

Definition of toxicity pathways associated with discrete initiating events has a variety of direct benefits and implications germane to the risk assessment process. For example, the ability to associate endpoints to one another through a continuum of biological organization (i.e., across molecular, cellular, target organ and apical endpoints) would be powerful, both for prospective and diagnostic risk assessments. In the former case, it would be possible to better link responses at intermediate biological levels of organization to both the initiating event and the adverse outcome. In the case of diagnostic assessments, delineation of toxicity pathways would contribute directly to understanding of the toxicological significance of alterations in markers of exposure based on changes in gene expression. From another perspective, knowledge of key initiating events relative to alterations in endpoints at higher levels of organization could enable a direct assessment of the technical validity of using mixture models based on similar versus dissimilar initiating events. In addition, identification of these events via alterations in gene expression could help in species extrapolation. Demonstration that toxic initiating events are similar across species would reduce uncertainty associated with extrapolation across species. Knowledge of common initiating events for a chemical or class of chemicals would focus the challenge of extrapolation across species on comparative dosimetry. One must always be aware, however, that species can display unique responses to the same perturbation, and while the initiating event may be identical across species, responses can diverge significantly such that different genes and tissues are affected in different species. Thus, the research focus may be on concordance of the initiating event rather than effect

or site of response.

Approaches used in computational toxicology will significantly improve our ability to understand and predict how xenobiotics may interact with biological systems. Figure 2 illustrates components of a toxicity pathway for some commonly used adverse outcomes in human health and environmental risk assessment. This schematic demonstrates the linkages between biologically effective concentrations of a chemical at a receptor/ligand site that lead to cellular and organ responses associated with an adverse effect at the individual level. These approaches can then be used for identifying and utilizing profiles of gene expression linked to cellular alterations and adverse effects or outcome.

5. Metabonomics

Metabonomics

Elucidate changes in metabolic patterns for range of endogenous metabolites
Generate NMR spectral profiles for chemicals
Build models to evaluate effect of novel chemicals on endogenous metabolites

Genomics and proteomics allow for the measurement of response to chemicals on the genetic and cellular protein level, respectively; however, neither provides a complete description of metabolism and chemical toxicity. For example, in some instances a xenobiotic may elicit changes in gene and protein expression, which are compensated for elsewhere, and result in no net change to the organism (i.e., no change in metabolite profile). To fully understand xenobiotic metabolism and toxicity in the context of genomics and proteomics, it is crucial to understand the metabolic status of the whole organism. The use of metabonomics provides such a means, i.e., it augments and complements genomic and proteomic responses to xenobiotic exposure, and provides a connection between genomics and proteomics with tissue function. The term "systeomics" has recently been coined to describe the integration of these fields. Metabonomics is the multi-parametric measurement of metabolites in living systems due to physiological stimuli, or genetic modification. The ability to conduct metabonomic-induced studies depends on the application of advanced analytical techniques such as high-resolution NMR spectroscopy and multi-variable statistical programs. ORD is in the process of purchasing a wide-bore 600 MHZ NMR for metabonomic analysis in support of the Computational Toxicology Research Program.

The application of metabonomics to toxicity testing involves the elucidation of changes in metabolic patterns associated with chemical toxicity based on the measurement of component profiles in biofluids (i.e., urine), cells, or tissues, and enables the generation of spectral profiles for a wide range of metabolites. NMR pattern recognition technology associates target organ toxicity with NMR spectral patterns and enables the generation of spectral profiles for a wide range of endogenous metabolites. Metabolite profiles (e.g., endogenous metabolites such as creatine, lactate, glutathione) could provide a measure of the real outcome of potential changes as the result of xenobiotic exposure.

The application of metabonomics can provide mechanistic information that could improve the risk assessment process. Assuming that groups of compounds induce similar changes in gene, protein, and metabolite profiles, it may be possible to classify compounds based upon their profiles. By including a large number of compounds with known toxicity in databases, ORD can build prediction models to compare and evaluate expression profiles of novel compounds. For example, association of a given toxic endpoint with a characteristic shift in cellular metabolites could provide a "fingerprint" that is characteristic of a specific mechanism of toxicity. Once a series of fingerprints are defined for different mechanisms, the metabolite pattern for a toxic chemical of unknown mechanism could be compared to the database. This could provide a very powerful tool for categorizing toxicants according to mode of action.

6. Systems Biology

Systems Biology

Computational models that reconstruct a cell, organ or organism's function from component parts
Allows validation and simulator experiments that build confidence in predictive ability of adverse effects

Conventional molecular biology strives to examine key events at ever increasing finer levels of detail. The Computational Toxicology Research Program, combined with the work being conducted by a number of outside organizations, will provide a wealth of information on the impact of toxicants by using genomic, proteomic, and metabonomic techniques. In order to be most useful, this information must be integrated together into a coherent picture. Systems biology is a new field of science that uses computational methods to reconstruct an integrated physiologic and biochemical model of an organism's or cell's biology. The approach is similar to developing a wiring diagram for a complicated electrical system or an engineering diagram of how a vehicle is put together and how the different parts interact and function together. In this regard, it is targeted at studying how normal biological processes are governed, and how alterations can lead to diseases or other unwanted outcomes. Understanding how a normal cell or organism works is key to understanding how toxicants can exert effects. For example, in developing a biologically based dose response model for the developmental effects of 5-fluorouracil, ORD researchers were able to describe the effect of this chemotherapeutic on thymidylate synthesis activity (its target enzyme), on subsequent nucleotide pool perturbations, on alterations in cell cycle times, and ultimately in the size of the fore limb bud. However, the investigators could not describe why the fourth digit was the most impacted, because not enough was known about the normal biology of limb development to understand how the preceding events altered the developmental programing. In this example, a systems biology approach is necessary to understand the underlying biology of limb development in order to better understand how the outcome of concern actually resulted from the precedent biochemical and cellular events. A systems biology approach will enable the integration of disparate data developed by biologists, computer scientists, chemists, engineers, mathematicians and physicists to construct models of organismal function and how they respond to a toxic insult. A choice has to be made about the scale and level of detail for each systems biology model and it is likely that models useful to the

Agency will be built upon individual subcomponents assembled into a larger scale system. Once these models are developed, then hypotheses can be developed and tested through virtual simulations prior to designing targeted experiments to validate and inform the models. An integral part of this Computational Toxicology Research Program will be the use of relevant model organisms to expand our understanding of the regulation of biological processes and how toxicants can perturb these processes. The goal is to improve risk prediction. In particular, cell signaling systems are receiving considerable attention in systems biology, and this might be a promising approach. An initial step to designing ORD's system biology efforts might be to organize a scoping workshop to help identify promising areas for research and development. To supplement its intramural efforts, ORD expects that the extramural grants [Science to Achieve Results (STAR)] program will be able to make contributions in filling the research needs presented by systems biology.

7. Modeling Frameworks and Uncertainty Analysis

The Computational Toxicology Research Program requires a modeling framework to develop a functional tool for prioritizing chemicals for subsequent screening and testing and enhance quantitative risk assessment. Modeling frameworks represent the software infrastructure required to facilitate modern environmental modeling. Modeling solutions to regulatory-based assessment needs require the development and application of science-based models and databases that span the source-to-outcome continuum. Modeling frameworks act as the computer operating system that contain and manage the coordinated execution and data exchange of numerous science-based models. They also facilitate access to external data sources, model output data analysis and user interfaces.

Modeling Frameworks and Uncertainty Analysis

Standardize format and interchange protocols for information generated by computer simulation
Technology for linking required databases
Perform uncertainty analysis methods analysis

ORD will design and implement a modeling framework (based on existing and proven technologies) that will standardize the format and interchange protocols for all information generated via computer simulation for the Computational Toxicology Research Program. The technology will contain (or access via internet) science-based models for simulating the environmental fate and transport of chemicals (transformation simulators), human and ecological exposure, the fate and transport of chemicals within human and ecological receptors (metabolic simulators and PBPK), toxicity pathways (QSARs) and finally adverse outcomes (systems biology models). In support of this modeling, the technology will include database connectivity tools for linkage to databases unique to the Research Program such as sequence databases, libraries of metabolic and toxic pathways, metabonomic profiles, and bio- and chemoinformatics.

In addition to providing the necessary modeling technology, ORD will also target the development of uncertainty analysis methods. As the scope of science needed to answer the broad questions posed by modern regulatory initiatives expands dramatically, our ability to quantify the accuracy of our model-based estimates decreases. A major focus of emerging scientific inquiry is related to the characterization and quantification of uncertainty in "high order systems." ORD has configured a supercomputer (a cluster of 150 PCs) to facilitate the distributed computing (parallel processing) necessary to execute sophisticated modeling simulations involving numerous models and databases. This hardware infrastructure is specifically designed to support the development a wide range of uncertainty analysis methods.

B. Provide Predictive Models for Hazard Identification

As discussed previously, there is a need by the Agency to develop predictive models for hazard identification. This section describes three areas where computational approaches are being considered or where such methods could have an impact.

1. QSAR and Other Computational Approaches

QSAR and other Computational Approaches

Quantifying physico-chemico parameters to predict fate
Identification of potential hazard in absence of empirical data
Prioritizing large groups of chemical for later testing
Framework for optimized use of "omic" data
Estimate missing parameters for untested chemicals

Like its physico-chemical properties, the biological activities of a chemical are the result of molecular interactions between the chemical and its immediate environment. When models for specific molecular interactions are developed, the activity of chemicals with respect to those interactions can be estimated directly from chemical structure. These QSARs serve as an important tool to screen untested chemicals for their potential to interact with hundreds of different environments using only the chemical structure and a virtual library of chemical and toxicological models. As such, QSARs have been used to optimize laboratory testing when the number of untested chemicals exceeds the resources available for testing. QSARs have also been used to provide estimates of missing data in lower tier risk assessment. In the case of the initial screening of chemicals for their potential to disrupt the endocrine system, models of molecular interactions with critical receptors and enzymes can be used to develop a series of computational methods to classify each chemical based on its likelihood of binding to a receptor or inhibiting a crucial enzyme. The intent of the QSAR screening is to offer a list of chemicals most likely to be positive in standard toxicity screening assays.

Another application of QSAR is to estimate the toxicity of untested chemicals directly from chemical structure. Some chemical properties and reactions can be directly related to

structural parameters and, for these, QSAR has in some cases been used as a cost-effective surrogate for routine laboratory measurements. For certain applications such as fate and effects modeling requiring expensive laboratory measurements of chemical properties, the structure-property relationships are now sufficiently reliable that QSAR estimates rather than measured values are widely used. QSAR can also be useful in estimating potency within a class of chemicals relative to acute toxicity endpoints.

Emerging "omics" technologies have excellent potential to generate information that will inform and improve the QSAR modeling process. Specifically, alterations in gene expression can be used to identify toxicity pathways and associated key molecular initiating events. Once initiating events are established for any given toxicity pathway/adverse outcome, predictive modeling can form the basis for dealing with large numbers of chemicals in a relatively rapid fashion. Specifically, there is little question that, given high-quality data sets, current QSAR modeling methods can effectively predict discrete biological phenomenon at the molecular level in terms of interactions of chemicals with different classes of lipids, nucleotides or proteins. Therefore, the key is defining the biological phenomenon for which data should be collected. If molecular initiating events can be identified relative to specific adverse outcomes, appropriate *in vitro* or *in vivo* assay systems (e.g., receptor binding assays) can be identified/developed to serve as a basis for generating the data needed for robust models.

Although the relationship between the generation of genomics information and QSAR modeling can be depicted in a linear fashion it is, in reality, a process that is iterative in nature, with multiple potential points of "entry" in terms of genomics or modeling components. At one end of the spectrum, if the initiating event through which a chemical elicits adverse effects is completely unknown, genomics approaches in which large numbers of expressed genes are assessed can be used in a "discovery" mode. This information may effectively identify the unknown chemical as similar to other previously-tested chemicals for which there is an understanding of toxicity pathways and associated initiating events. Alternatively, the genomics information may serve as the basis for defining previously un-characterized initiating events which would serve as the starting point for delineating new/alternate toxicity pathway(s). As key initiating events are identified, appropriate data "generation" assays can then be developed to generate data for QSAR and other computational models capable of predicting either short-term (acute) or chronic toxicity.

2. Pollution Prevention Strategies

In support of pollution prevention strategies, ORD is developing methods to estimate the potential environmental impact of chemicals that are released into the environment. These methods are used to evaluate chemicals for potential harm both to humans and the environment in a life-cycle

Pollution Prevention Strategies

Methods to estimate potential impact after release into environment
Final indicators to compare large numbers of chemicals

assessment framework. These chemical evaluations are performed over a wide range of environmental impact categories, including human health (acute, chronic, and carcinogenic indicators), aquatic health (acute and chronic indicators), terrestrial health, global warming, ozone depletion, smog formation, acid rain, eutrophication, and natural resource depletion. To this end, several pollution prevention tools have been developed for a variety of uses. Depending on the level of analysis desired, the complexity of the model for evaluating human and ecological health concerns will dictate the type of data required. These data may be collected from acute toxicity studies, detailed systems biology models, fate and transport models, exposure models, or chronic toxicity studies. Regardless of the level of sophistication in the models, the final impact indicators (e.g., a broad range of mid-point effects or final outcomes, such as human deaths, human illnesses, crop damage, water quality issues, air quality issues) could be used to compare a large number of chemicals. QSARs have also been developed by ORD to provide estimations for toxicity values for those chemicals which have no experimental toxicity data. For example, QSARs can be used to estimate endpoints, such as the 96-hr LC_{50} values for fathead minnows and the oral rat LD_{50} values. These estimated values can then be incorporated into various environmental models and used in pollution prevention strategies..

The research that is being proposed for this part of the Computation Toxicology Research Program will focus on improving the models and estimations that are currently being used in the aforementioned pollution prevention tools. For instance, these tools will greatly benefit from increased certainty in the fate and transport models (as discussed in Section II.A.1.a); they will greatly benefit from a better understanding of metabolic maps of chemicals (as discussed in Section II.A.1.b). These tools will also benefit from the enhancement of the quantitative risk assessment process, such as increased knowledge of dose-response assessments (as discussed in Section II.C.2) and of cross species extrapolations (as discussed in Section II.C.2). These pollution prevention tools are currently being used in academia and industry as well as by the program offices.

3. High Through-Put Screening

High Through-Put Screening

Vast chemical inventory not tested
Rapid, efficient means to provide preliminary data
Recommended for Endocrine Disruptors (EDSTAC)

Applications of new molecular and other technological advances hold promise for the development of high through-put screens (HTPS). It has been suggested that HTPS be used a rapid, efficient means to provide preliminary endocrine effects data on chemicals considered in the endocrine disruptors screening and testing program. In view of the estimated 87,000 chemicals under consideration, it would be beneficial if rapid, HTPS systems could be developed to assist in prioritization of chemicals for further testing. Since all processes are automated and can be programmed to run continuously, large numbers of samples can be screened in a relatively short period of time using this technology. New approaches have the potential for making significant advances over existing EDC screens in terms of speed, high-

throughput capability, sensitivity, reproducibility, and reduction in animal usage in a screening and testing program. HTPS will be a valuable tool to help elucidate and characterize toxicity pathways (see section II.A.4). Approaches under development could focus on: classic ligand-steroid receptor-coregulator/cofactor interactions; non-genomic mechanisms of steroid hormone action; or mechanisms involving synthesis, metabolism, or degradation of estrogens, androgens, and thyroid hormones. Furthermore, some HTPS approaches may be flexible and versatile enough to allow for screening to be carried out across vertebrate classes which could be valuable in helping address cross-species extrapolation issues (see section II.C.2.b).

C. Enhance Quantitative Risk Assessment

The previous section discussed how computational toxicology might be used to screen chemicals to identify candidates for toxicity testing. This section discusses applications to the Agency's quantitative risk assessments, which typically estimate either a risk of adverse effect or a toxicity benchmark level such as a reference dose or a reference concentration depending on the endpoint being assessed. Computational toxicology has the potential to enhance the Agency's current risk assessment methods and contribute to the development of new methods that are consistent across endpoints and species. One aspect of the ORD program will be to develop broadly applicable risk assessment methods that take advantage of new technologies and the data generated by the Computational Toxicology Research Program.

1. Applying Computational Toxicology in Quantitative Risk Assessment

ORD has ongoing research in two areas that can be related to risk assessment, including computational chemistry (QSAR) and mathematical biology [PBPK/Biological Based Dose Response (BBDR) modeling]. The Agency's Program and Regional Offices are faced with hundreds of site-specific risk assessments for which some estimate of risk is essential to inform Agency priority-setting. Many of the site-specific environmental issues involve chemicals for which there are insufficient data. ORD is currently exploring the use of QSAR to estimate toxicity benchmarks for such chemicals. ORD also has an ongoing program to apply PBPK and BBDR models to risk assessment. In particular, ORD envisions a systems biology approach to dose-response modeling, integrating PK and PD. QSARs can be used to estimate parameters of PBPK models and cellular response. ORD is developing such models and linking them to systemic (whole-organism) dynamics in order to estimate the toxicity metrics needed for risk assessment. ORD is also exploring ways to use more mechanistic approaches to dose-response assessment that will lead to human health risk assessment approaches that are consistent across all endpoints, replacing the assumption that some endpoints are non-threshold effects (cancer) and other endpoints are threshold effects. ORD is also exploring data-based approaches to adjusting default uncertainty factors in current non-cancer risk assessments. As a first step, ORD is exploring the use of PBPK data and models to adjust default uncertainty factors used in inter- and intraspecies extrapolation and for developing an assessment of target organ dose for use in dose-response assessment of mixtures. Similar efforts for the PD uncertainties would be the next step.

In the field of computational biology, the Agency is just beginning to consider application of genomic/proteomic data to risk assessments. The Agency's Interim Genomics Policy outlines the current state of the application of genomics data (which is defined to include proteomics and transciptomics) in risk assessment. Genomics data may be considered in Agency decision-making but they are insufficient, standing alone, to inform decisions about environmental risk. It is essential, therefore, that the Agency consider how the information generated by this new technology will be utilized in human health and ecological risk assessment. There are many issues that need to be considered before routinely adopting and/or replacing existing data requirements with expression data for Agency risk assessments. One goal of the Computational Toxicology Research Program is to address these questions in order to develop methods for use of computational toxicology in quantitative risk assessment.

2. Examples of Applications of Computational Toxicology to Quantitative Risk Assessment

There are many potential applications of computational toxicology in quantitative risk assessment. The following sections discusses three areas, including dose-response assessment, interspecies extrapolation, and toxicity of mixtures.

a. Dose-Response Assessment

Genomics/proteomics technologies have important implications for health and ecological risk assessment, and there is a need to develop methods that use these data to improve quantitative dose-response assessment. One important area is the use of emerging technologies to determine the shape of the dose-response curve in the low dose range, based upon *in vivo* and *in vitro* data which can be shown to be correlated with low dose adverse effects. Studies using emerging technologies may also result in the identification of useful (simple, sensitive and relevant) biomarkers of effect so that they can be used in dose response, not just hazard identification, studies to more accurately diagnose effects in the low dose range, and for compounds with weaker potencies.

COMPUTATIONAL TOXICOLOGY AND QUANTITATIVE RISK ASSESSMENT

Defining the shape of the dose response at low exposures using molecular indicators of response
Developing biomarkers for use in analysis of low dose responses
Validating the interpretation of molecular indicators of response
Defining the relevance of modes of action for risk assessment
Constructing BBDR models of high priority outcomes
Assessing population level effects in ecosystems
Cross-species extrapolation
Assessing toxicity of mixtures
Integrated human and ecological risk assessment

Research from the Computational Toxicology Research Program may also lead to the identification of biological effects that could be used as the adverse effect for risk assessment. For example, if fetal testis endocrine function is altered such that steroid hormone production and insl3 gene expression are reduced by 50% or greater, it is possible that this change could be considered an adverse effect.

Chemicals producing such effects might then be regulated on this information alone. The assumption of such an approach is that reductions in insl3 are always associated with developmental malformations so there is no need to continue to demonstrate this for every chemical. Chemicals could then be regulated on the basis of fetal endocrine effects alone. The Agency has used endocrine data in this manner on occasion, but the approach would be strengthened by including genomic or proteomic information to support the hypothesis that a specific pathway had been sufficiently disrupted by a chemical such that adverse effects would definitely result later in life.

Another application of emerging technologies from the Computational Toxicology Research Program would be a determination of the relevance of the mechanism or mode of action of low dose adverse effects to humans and other species using *in vitro* and *in vivo* approaches. Such research could address the critical pathway initially involved in chemically-induced adverse effects and how well the mechanism is conserved among mammals and other vertebrates.

Although scientists both inside and outside of the Agency have been proposing the application of BBDR models in risk assessment to reduce uncertainties in the process for several years, this goal has not been realized in spite of several long-term research efforts due to the complexity of the models. One question that could be addressed by the Computational Toxicology Research Program is how quantitative mechanistic genomic data could be used to develop BBDRs that produce realistic values for risk assessment.

b. Cross -Species Extrapolation

Cross-Species Extrapolation

Based upon knowledge that all species arise from common evolutionary ancestor
There is varying degree of homology among animals

One of the major challenges in regulatory toxicology is the prediction of toxicity of a chemical(s) or classes of chemicals across species. In its risk assessments, the Agency often predicts possible effects in humans from studies in rodents and other mammalian test species, while in ecotoxicology, extrapolations to literally thousands of other species are typically based upon results of assays with a handful of surrogate test organisms. In addition, it is generally assumed that adverse effects seen in vertebrate wildlife are relevant to other species, including humans. For example, when fish display evidence of exposure to estrogens or androgens in the environment, or frogs exhibit limb malformations, there are immediate concerns about potential impacts to humans and other wildlife. The concept of interspecies extrapolation is based upon the knowledge that all species arise from common evolutionary ancestors and, hence, there is a great deal of homology among animal species with regard to basic biological pathways. However, the ability to extrapolate from species to species is not trivial for two reasons. First, exposures to chemicals vary as a function of an animal's physiology and its environment. For example, humans are not exposed to estrogenic or androgenic materials in effluents to nearly the same degree as fish living in, or adjacent to an effluent. In a

more phylogenetically similar comparison, the Florida panther is at greater risk for exposure to (and effects of) bioaccumulating contaminants than are other populations of panthers because it feeds at a higher trophic level than other populations, not because of genetic differences.

Another aspect of differential dosimetry among animals is metabolism of xenobiotics. Pathways of metabolism can differ significantly across species, even in closely related animals. In fact, in many cases, failure to metabolically activate a chemical or conversely, its rapid detoxification, is a primary basis for the lack of response of an animal, not because of species-specific differences in gene expression and subsequent toxicity. For example, the anti-androgenic action of vinclozolin or the estrogenic activity of methoxychlor require metabolism to active metabolites. Testing either of these chemicals in a species that does not produce "active" metabolites could lead to the mistaken assumption that they would not affect endocrine function. This component of species extrapolation, that of comparative dosimetry, can be addressed using BBDR models that focus both on concentrations of parent chemicals and/or metabolites in target tissues. This type of modeling is an integral part of the Computational Toxicology Research Program.

The second challenge to species extrapolation involves how an animal actually responds to a given dose of chemical(s) of concern. Specifically, although there is remarkable similarity in basic biology among animals, there are also significant species-specific differences in genes, proteins, biochemistry and physiology. These differences lead to uncertainties in interspecies extrapolation such that toxicologists are generally more comfortable extrapolating among closely related species than among those that have been separated phylogenetically for a longer period. To address this aspect of species extrapolation, definition of toxicity pathways from a comparative perspective is critical; as such, the Computational Toxicology Research Program will focus on extrapolation. Specifically, characterization of toxicity pathways in well-defined animal models serves as the basis for identifying key control points (e.g., receptor -mediated signaling) that, quite possibly, would be conserved across species. This type of conservation would be expected for many receptor-based processes (as illustrated by estrogen- and androgen-controlled pathways). When control points are known, state-of-the-art molecular biology (e.g., genomic) techniques can then be used to assess the degree to which extrapolation of effects associated with chemicals with specific mechanisms of action can be supported. For example, once it has been demonstrated that receptor activation by xenobiotics is key to eliciting toxicity, it is then possible to focus on comparative receptor binding studies across species as a basis for extrapolation. The critical point here is that, if pathways can be reliably defined in representative model species, it is not imperative to know a comparable amount of toxicological information in untested species, only whether the latter possess key components of pathways of concern (and how these components are affected by xenobiotics).

c. Chemical Mixtures

Chemical Mixtures

FQPA specifies that cumulative effects must be addressed for exposure to multiple chemicals acting by a similar mechanism

Lack of information regarding mechanism or mode of action for risk assessment of mixtures

The Agency's guidance on risk assessment of mixtures indicates that multi-chemical exposures are ubiquitous, including air and soil pollution from municipal incinerators, leakage from hazardous waste facilities and uncontrolled waste sites, and drinking water containing chemical substances formed during disinfection and that exposure scenarios are very diverse. Two approaches are generally used to assess exposure to and effects from mixtures, i.e., study of whole mixtures or individual mixture components. The Food Quality and Protection Act (FQPA) specifies that cumulative effects be addressed for exposure to multiple chemicals that act by the same mechanism. However, a number of uncertainties exist regarding the level of mechanistic similarity among chemicals. Considerable complexity arises even when examining interactions among chemicals that have "estrogenic" activity. While whole mixture studies offer environmental relevance, it has been recommended that whole mixture screening go forward only after screening had been conducted for a number of individual chemicals. Clearly, mixtures research on exposure to and effects from xenobiotics on humans and wildlife is open to reductions in uncertainty through computational toxicology research. Mixture research over decades is poised to take advantage of unifying principles from shared chemical and biological mechanistic research. New tools developed in the "omics" and computational methods fields show huge potential for employing molecular profiling to understand the complexity of mixture exposure and elucidating the mechanisms underlying biotransformation, uptake, distribution and response. Technological advances now enable study of the joint and interactive properties of mixtures and definition of those characteristics that are sufficiently similar to allow extrapolation of data from one mixture to another. They have the potential to allow identification of emergent properties of real-world mixtures of xenobiotics at environmental exposure concentrations rather than from defined mixtures at high (toxic) concentration. Hypotheses can be tested to identify mixture classes (by activity or structure) that are amenable to component approaches. Mixtures assessment guidelines stand to become much more uniform, relevant and easily applicable to risk assessments of real world exposure scenarios.

III. CURRENT ACTIVITIES

The previous section described several areas in which a program on computational toxicology could provide methods and models that would lead to more efficient ways to assess chemicals for screening and testing, as well as improve quantitative risk assessment. Section III demonstrates that ORD currently possesses the capability to utilize approaches necessary for the ultimate development of a Computational Toxicology Research Program in the future. The work described below is divided into three categories, including ORD's initial research to demonstrate the feasibility of the computational toxicology concept, examples of on-going research, and linkages to external research groups.

A. Proof-of -Concept: Endocrine Disrupting Chemicals (EDCs)

Proof-of-Concept Studies with EDCs

Refined QSAR models
In vitro models
Toxicity pathway characterizations

Initiated by a FY02 Congressional mandate to explore alternatives to the use of animals in toxicological testing, ORD began a research effort to explore the use of emerging technologies and computational approaches to better prioritize chemicals for screening and testing. Because much is known about how EDCs interact with biological systems to cause adverse health, it was decided to focus the program on this class of chemicals and to conduct several feasibility proof-of-concept experiments to determine the feasibility of using computational toxicology approaches to meet an immediate Agency need. Understanding the key biological pathways impacted by endocrine disrupting chemicals affords the opportunity to design approaches that are more efficient in terms of resource utilization. It also allows the Agency to extrapolate findings from a smaller set of chemicals to the broader chemical universe using the tools of computational chemistry. Thus, projects exploring *in silico*, *in vitro* and *in vivo* approaches could facilitate both the prioritization of chemicals for screening, reduce the need for some *in vivo* assays, and provide *in vivo* assays that have a greater breadth of coverage of endocrine alterations and/or provide better predictiveness of potential adverse health outcomes. The overall program is intended to have both short-term, intermediate, and long-term goals. The efforts in this proof-of-concept activity is directed at developing better tools and assays for which to monitor selected aspects of endocrine disruption, and success will be measured against the recommendations put forth by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC). Success would provide us confidence that the approaches would be applicable to others modes of toxicity where the underlying biology is not so well understood at the present time. Descriptions of the activities follow:

Receptor Binding Models - The EDSTAC recommended approach to screening chemicals for endocrine activity contained an element that proposed the use of QSAR models of receptor binding to help prioritize chemicals for further screening. EDSTAC also recommended that the Agency undertake a study to evaluate the *a priori* predictions of available QSAR models for estrogen receptor interaction by obtaining data on competitive binding affinity for the estrogen receptor from a single laboratory using a standardized protocol on approximately 300 chemicals. This demonstration exercise found the models needed additional work to improve their sensitivity, specificity and predictive capabilities before they could be used in a regulatory context. The lack of complete agreement between binding information and the QSAR model appears to be related to inconsistent receptor-binding information in the training sets used to initially construct the QSAR models. The values to derive the model were derived from multiple laboratories, and based on IC50 values, which potentially can introduce errors because non-competitive binding might be present. Therefore, under the proof-of-concept effort, ORD is taking 70 of the 300 chemicals that showed some evidence of receptor interaction and generating K_i values for each of them. This will

provide and unbiased and unequivocal measure of receptor binding. Following acquisition of these data, the QSAR model will be re-derived using the new training set and its predictability will again be assessed. This effort will demonstrate how international criteria for transparency, domain coverage, and model acceptance evaluations can be met, while reducing the number of chemical tests needed when compared to random chemical testing approaches. Through this iterative process, ORD will test the hypothesis that given a robust data set of high quality, reliable QSAR models can be developed and used in hazard identification. Assuming success in this effort, other elements are focusing on the androgen receptor (AR), but here more fundamental work is also needed to better characterize, for example, the ligand binding site.

In vitro models - Knowing the pathway that is of concern for toxicity in whole animals should allow the development of simpler *in vitro* systems that can provide quick and inexpensive evaluation of the potential for chemicals to interact with that pathway. EDSTAC raised concerns about the need to evaluate the effects of chemicals on the steroidogenic pathways in Tier I screening approaches. EDSTAC addressed this data gap by recommending a combination of studies in pubertal rodents and in enzyme inhibition studies on either placenta or minced testes preparations from male rats. Both of these approaches have a number of limitations for their ability to study the synthesis of the key steroids (estradiol and testosterone) from cholesterol, including the fact that they are more targeted at detecting inhibition of synthesis rather than enhanced synthesis. A human cell line (the H295 line) has been identified that maintains ability to synthesize estrogen from cholesterol, and therefore addresses many of the limitations of the EDSTAC approach. Under the proof-of-concept effort, ORD is developing standard operating procedures for use of the H295R cell line to evaluate each step of steroidogenesis at the genomic, proteomic, and metabonomic level. ORD will compare the results from a set of chemicals with the EDSTAC-recommended assays to determine if this cell line affords more powerful, yet easier to achieve, answers to the potential to alter steroidogenesis.

Toxicity Pathway Characterizations - These studies are integral to the proof-of-concept effort and will consist of studying thyroid gland functioning in ecologically relevant species and by examining the integrating function of the vertebrate hypothalamic-pituitary axis in responding to the presence of EDCs.

The thyroid gland was selected as a target for the effort because it represents an endocrine organ whose function is disrupted not by direct xenobiotic interaction with the thyroid receptor, but by interactions elsewhere in the endocrine loops (e.g., iodine uptake processes, hormone synthesis, hormone modification, and hormone metabolism). Given the fact that thyroid function can be perturbed at different points in the thyroid pathway, research is being directed at developing a suite of endpoints that could ascertain which toxicity pathway is initiated by a specific chemical. Each unique site of chemical interaction with the thyroid signaling axis would be considered a separate toxicity pathway, given that the chemical structural requirements for interaction with each site are likely unique. This would facilitate the development of QSAR predictive models for each toxicity pathway. Therefore, a distinction among the multiple toxicity pathways that all impact the central thyroid hormone signaling axis would be advantageous as a way to think about risk assessment applications where chemical prioritization for further testing is the goal. It would also be

advantageous to maintain the multiple toxicity pathway distinction when assessing aggregate vs cumulative chemical risk. A common mechanisms of action could not be assumed unless it is shown that mixtures of chemicals across these different toxicity pathways exhibit strictly additive behavior when equal toxic units are combined for joint toxicity assessments. Success here would demonstrate that a more global focus on endocrine function using genomic approaches and critical life stages can provide a more meaningful read out that the EDSTAC-recommended amphibian metamorphosis test, which is neither highly sensitive nor very informative as to disturbances in the function of the thyroid.

The last area covered by research on toxicity pathways is more exploratory in nature, i.e., involves exploring the possibility that through the use of genomic and proteomic evaluations that a *single in vivo* test for endocrine disruptor activity can be developed based on the rationale that the central nervous system (CNS) contains all of the relevant receptor and enzymatic target sites of interest. Taking advantage of the fact that there is a wealth of information available concerning the physiological regulation of the thyroid, adrenal and gonadal axes by the hypothalamus and the pituitary glands, it may be possible to test empirically the extent to which changes in these endocrine systems are sensed and responded to at the level of the CNS. It is anticipated that the development of "genomic response profiles" following exposure to EDCs of known action will provide the means to identify the target pathways that lead to altered reproductive/thyroid/adrenal function. This approach, if successful, would be superior to the current proposed male and female pubertal assays as it could predict all target pathways whereas the current assays can not necessarily identify specific CNS target sites. Furthermore, this approach has the potential to go beyond screening and may incorporate elements important to establishing efficient Tier 2 tests.

The concepts under study in the current computational toxicology proof-of-concept projects, using EDCs as an example, will be applicable to future studies on the potential of non-endocrine disrupting chemicals to affect other biological systems and signaling pathways. Computational toxicology proof-of-concept research includes the following components: 1) elucidation of pathways of chemical toxicity, from initiating event to adverse outcome in individuals or populations; 2) identification of key assays indicative of toxicity pathways that provide a means to efficiently, wisely, and with minimal animal testing, extrapolate across chemicals and species; and 3) the application of an iterative QSAR strategic test design, where models are developed and strategically improved, with minimal testing, until criteria for regulatory acceptance are met. The predictive models developed under this approach thus have a solid foundation in scientific principles and will provide the Agency with defensible approaches to priority-setting.

B. Internal Linkages

There are several on-going research projects in ORD's core and problem-driven research program that are supportive of efforts in computational toxicology. ORD's core research program aims to provide broad, fundamental scientific information that will improve understanding of problem-driven human health issues arising from risk assessment in the Agency's Program and

Regional Offices. Several projects in ORD focus on elucidating toxicity pathways for adverse effects having a high regulatory impact, i.e., cancer, reproductive, pulmonary and neurotoxicity. Problem-driven research focuses on issues related to specific contaminants, e.g., particulate matter, arsenic, disinfectant by-products (DBPs), and EDCs. As examples, the two following sections describe on-going research on the human health effects of DBPs and the assessment of an aquatic species in ecological risk assessment. Appendix A provides additional examples of ORD projects that support the Computational Toxicology Research Program.

1. Human Health Research

Under the Information Collection Rule, DBP occurrence data for major water systems across the country are becoming available, and data are being collected on DBPs that have been predicted to have an adverse health effect but have little or no previous quantitative occurrence information. In addition to this utility-based information, data on individual water use and biomarker development will increase the precision of exposure assessment. These data will help to identify classes of DBPs and candidate model compounds that are present at the greatest concentration. DBP concentration at the tap can vary widely and be dramatically different from reported averages from water utilities so a series of measurements on drinking water at different points in the distribution system would provide the necessary information for better informed exposure models. Currently exposure modeling from ingestion of disinfected drinking water does reflect the general characteristics of the source water and disinfection process. However, proposed work in developing improved exposure modeling will result in a significant refinement of these models so that more quantified data on DBP component analysis can be included to allow more accurate prediction of the concentration of the most toxic DBPs based on the characteristics of the local water. This work will then be combined with the response models to develop a computational approach for predicting potential adverse effects based on the predicted and actual characteristics of the mixture of DBPs.

Computational chemistry methods and molecular modeling approaches are being employed to compute a variety of structural, electronic and reactivity characteristics of DBPs, their postulated metabolites and/or adducts for input and consideration in the development of structure-activity models. Efforts currently focus on differences based on the bromine content within a class of DBPs. The central issue, in this case, is the role of bromination in determining and modulating biological activity within these classes. Toxicity information, in conjunction with known principles of organic chemistry of halogen-facilitated reactions, will be applied to understand the possible range of mechanism-based reactivity and how

Examples of Chemicals Under Study by ORD

Arsenic
Disinfectant By-Products
Endocrine Disruptors
Particulate Matter
Pesticides

these are modulated by chlorination and bromination. These modulations of activity include DNA reactivity, adduct formation, and carcinogenicity. Computational chemistry and SAR modeling will be used to prioritize and aid in the preliminary hazard assessment of DBPs for which little or no toxicity data are available. These approaches can also be used to generate mechanism-based SAR hypotheses pertaining to classes of halogenated drinking water contaminants and help guide the design of experimental studies to most productively address areas of greatest uncertainty.

2. Ecological Research

ORD has made a significant commitment to genomic and proteomic research on model aquatic vertebrates. For example, ongoing work in this area has focused on the use of genomics and proteomics to delineate toxicity pathways associated with disruption of key elements of the thyroid system in the amphibian model, *Xenopus*. Other work in this area is focused on small fish models, including the sheepshead minnow, medaka and the fathead minnow. A primary goal in the fish research is elucidation of mechanisms of toxicity induced by perturbation of processes controlled by the hypothalamic-pituitary-gonadal/thyroid (HPG/T) axes. The fathead minnow, *Pimephales promelas*, is an important model for this research since: (1) it is the Agency standard for teleost aquatic toxicity testing, (2) the fathead minnow 21-day reproductive toxicity test is the priority Agency-recognized screen for endocrine disruptors in teleosts, (3) significant progress has been made in gene discovery and initial cDNA microarray synthesis via core microarray facilities, (4) high-throughput sequencing of cDNA libraries by the Department of Energy (DOE) is likely, and (5) a multi-laboratory effort is enabling integration of exposure and effects research. Ultimately, identification of initiating events or other critical pathway elements will enable the development of QSAR models, initially for estrogens and androgens in fish, and subsequently for other targets on the HPG/T axes. As research focused on toxicity pathways associated with these axes develops, classes of chemicals that operate via other modes/mechanisms of action also will be considered; an important group of chemicals currently under consideration in this regard are polyfluorinated surfactants.

Besides the primary objective of toxicity mechanism elucidation, this collaborative research aims to significantly support other goals of the Computational Toxicology Research Program. For example, the discovery of genes specifically induced/repressed by hormone agonists and antagonists will enable the development of specific diagnostic molecular indicators of exposure and the linkage of PB/PK models to mechanistically-based QSAR models (see section I-C). In addition, genomic and proteomic research will provide the basis for comparison of toxic mechanisms of action across species. For example, the present ORD collaboration has already produced highly significant data on the conservation and function of the estrogen receptor across genera.

C. External Linkages

ORD is actively seeking to establish links with groups outside the Agency that have

expertise and capabilities that could complement and augment the emerging program on computational toxicology.

1. Chemical Industry Institute for Toxicology (CIIT) Centers for Human Health

ORD and CIIT have agreed to a memorandum of understanding (MOU) to advance the state-of the-science of computational toxicology. Among the goals of the agreement is the utilization of the complementary capabilities and expertise of ORD and CIIT in developing and applying computational toxicology approaches to human health risk assessment. Research activities common to both organizations focus on the use of computational methods and molecular biology toward the characterization of risks of environmental contaminants. CIIT focuses on the development of PBPK and PD, while ORD contributes to the joint effort by studying relevant toxicant-induced effects in various target organs (i.e., research on toxicity pathways). Improving the risk assessment process has been an emphasis of both groups for many years and approaches involved in computational toxicology are compatible with the goals of both organizations. This collaboration will foster exchanges of information, training opportunities, and technologies. Initial research will focus on examining the toxicity pathways for the effects of dibutyl phthalate in the developing testes.

2. Department of Energy (DOE)

In the Fall of 2002, ORD scientists visited Sandia and Pacific Northwest National Laboratories of DOE, which led to the development of 23 possible project areas thought to be suitable for possible collaboration. High priority projects of mutual interest to DOE and the Agency include: research to develop computational screening techniques beginning with traditional QSAR screening of chemicals to predict toxicity of EDCs; the development of new techniques to specifically identify molecular structures associated with toxicity; use of innovative and proteomic techniques to identify, characterize and classify sensitive subpopulations based on biological factors to reduce uncertainty in risk assessment; and characterization of atmospheric pollutants to better understand the processes influencing human risks to atmospheric pollutants, identify and characterize the casual agents associated with these effects, and better understand the systems biology and mechanisms associated with the exposure-to-dose-to-effects paradigm. A MOU and an Interagency Agreement has also been developed between DOE and ORD to provide High Performance Computing consulting and/or access to DOE non-classified computing equipment. In January, 2003, ORD scientists visited the US DOE Joint Genome Institute (JGI), which resulted in JGI's agreement to sequence the genome of selected species currently being used for testing and screening of environmental pollutants (i.e., *Pimephales promelas* and *Xenopus tropicalis*). Sequencing the genome of these test species could lead to molecular-based models for predictive toxicology. Other collaborations with genomics and bioinformatics experts at the University of Cincinnati Children's Hospital Research Center have begun to yield synthesis of complementary DNA microarrays with fathead minnow gene sequences and expressed sequence tags. These researchers represent the conduits for synthesis of large scale microarrays using sequences gleaned from the JGI collaborations.

3. National Institute for Environmental Health Sciences (NIEHS)

The National Center for Toxicogenomics (NCT) at NIEHS was formed to facilitate development of gene expression and proteomic methodology, create a public database relating environmental stressors to biological responses, collect information relating environmental stressors to biological responses, collect information relating environmental exposures to disease, develop improved use of computational mathematics in understanding responses to environmental stressors, and identify biomarkers of disease or exposure to enhance environmental health. Many of these objectives overlap with current core and problem-driven research at ORD. Preliminary discussion have been held with NCT concerning the Computational Toxicology Research Program at ORD. One possibility for collaboration is that the genomic and proteomic information from ORD's program on computational toxicology will be made available to the Chemical Effects in Biological Systems (CEBS) knowledge base at NCT. This database is a relational and descriptive compendia of toxicologically important genes, groups of genes and mutants and their functional phenotypes. This platform allows searching for information about the biological effects of chemicals and other agents and their mechanism s of action based on information from the literature and contributions from intramural and extramural sources. The database utilizes standardized procedures, protocols, data formats and assessment methods to ensure that data meet a uniform level of quality. It is expected that ORD will play a role in helping to build a publicly accessible toxicological database that will be capable of predictive toxicology and serve as a major resource for researchers to pose and test mechanistic hypotheses.

4. Science to Achieve Results (STAR)

Through its external grants program, ORD is developing a series of requests for assistance (RFAs) that will lead to the support of scientists in academic and not-for-profit institutions in research areas that complement the in-house research activities. Most of the support will be in the form of grants, but in some cases where ORD scientists would like to work more closely with the awardees, cooperative agreements may be awarded. The selection of both awarded grants and cooperative agreements will be through a competitive process. All awardees will be asked to participate in periodic progress reviews where intramural and extramural scientists are brought together to share and review data.

IV. NEXT STEPS

A. Review of the Framework

The next step in the development of a Computational Toxicology Research Program in ORD is to obtain external peer review of the *Framework for the Use of Computational Toxicology in Risk Assessment.* The first step in this process will be to consult with the Agency's SAB, and a meeting

with the SAB has been set for early September, 2003. Following this review, scientists external to the Agency will be contacted to provide a peer-review of the concepts and approaches in the framework. In addition, a workshop will be held September 29-30, 2003, to discuss how ORD will use the data and experience from other research organizations. In FY04, ORD will initiate a Request for Proposals to identify themes and intramural and extramural research approaches in the area of computational toxicology based on the priorities and process described in the following two sections. This process will lead to the development of an ORD Multi-Year Plan on Computational Toxicology that will describe the critical milestones this area of research will accomplish over the next 5-8 years.

B. Priorities for Research on Computational Toxicology in ORD

In developing a Computational Toxicology Research Program, the following decision-making criteria will be used in setting priorities for research:

1. Risk-Based Planning: Research should address an element in the source-to-outcome paradigm and be designed to improve quantitative risk assessment or facilitate the development of screening or testing strategies for chemicals.
2. Utilizes New Technology: Research using emerging proteomic/genomic methods is encouraged.
3. Hypothesis-Driven: Research to test hypotheses is encouraged.
4. Scientific Excellence: The quality of the science selected for support is critical for regulatory acceptance of the approaches to be developed by the Computational Toxicology Research Program.
5. Programmatic Relevance: Research should address a key Agency-related mandate concerning protection of human health and/or the environment.
6 Other Sources of Data: Research involving partnerships and collaborations with other organizations outside of the Agency is encouraged.
7. Capabilities and Capacities: It is important that the research can be completed within a reasonable period of time using available material and human resources.
8. Sequence of Research: It is likely that there will be critical, key steps that must be accomplished before other steps can be taken in the development of a research program in computational toxicology.

C. Process for a Research Program on Computational Toxicology

The development of an ORD program on computational toxicology will require coordination and communication among ORD managers and investigators and the continued involvement of the Computational Toxicology Technical Writing Team to ensure that the research is relevant, timely, and defensible. Efforts will be made to optimize existing intramural research by seeking out complementary extramural research efforts.

Following approval of this *Framework for a Computational Toxicology Research Program in*

ORD, the technical writing team will evolve into the ORD Computational Toxicology Research Steering Group. This group will provide oversight of the process to implement the Computational Toxicology Research Program, including the developing of an ORD Multi-Year Plan on Computational Toxicology to guide and coordinate research in this area. Scientist-to-scientist meetings involving intramural and extramural scientists will also be sponsored by the Steering Group to help integrate research with external groups working in this area, as well as within ORD. Such meetings will also help guide the research program in new directions as results become available. Periodic review of on-going research by the Steering Group will be important to demonstrate progress and design future approaches. Systematic communication with the Agency's Program and Regional Offices through the planning process will be important to provide them with an understanding of the nature and extent of the application of computational toxicology approaches to meet the Agency's needs to improve quantitative risk assessment and develop a strategy for hazard identification.

APPENDIX A

Examples of Current ORD Projects Associated with Computational Toxicology	
A. Improve Linkages in the Source-to-Outcome Continuum	
	1. Chemical Transformation and Metabolism Fate models- Core research to elucidate and model the behavior of organic contaminants in natural and impacted ecosystems and in complex biological systems (NERL) Fate models- Problem driven research to develop the methods, tools and databases to forecast the fate of pesticides and toxic chemicals during the drinking water process (NERL)
	2. Development of Diagnostic/Prognostic Molecular Indicators Developmental Biomarkers- core research that focuses on identifying molecular markers of developmental toxicity related to growth and maturation of organ systems (NHEERL) Virulence Potential-core research to develop molecular methods to measure virulence of microbial pathogens (NERL)

	3. Dose Metrics Cumulative Risk for Drinking Water Contaminants-problem-driven research to use QSAR, mixtures toxicity approaches and PBPK modeling to develop a cumulative risk method based on doses in target tissues (NCEA) PBPK Models in Fish-core research to use genomic data to validate output of PBPK models (NERL)
	4. Characterization of Toxicity Pathways Cell Signaling- core research to determine role of signal transduction pathways in toxicity pathways for high priority environmental chemicals (NHEERL) Framework for Defining Model and Mechanism of Action for Cancer and Noncancer Endpoints:-research to explore using genomics and proteomics as an approach for understanding mechanisms and the implications for risk assessment (NCEA)
	5. Systems Biology None
B. Provide Predictive Models for Screening and Testing	
	1. QSAR Approaches Application of QSAR and modeled exposure estimates in risk-based chemical ranking model (NCEA) Perfluorooctane sulfonate (PFOS)- problem driven research on MOA of PFOS, a breakdown product of several widespread and persistent chemicals in the environment (NHEERL)
	2. Pollution Prevention Strategies Endocrine Disrupting Chemicals Replacement Program - problem driven research to develop a software tool that will allow users to quickly identify possible replacements for chemicals that are known or projected to have endocrine disrupting potential (NRMRL) Pollution Prevention Tools - core driven research to incorporate results of other computational toxicology projects into software tools used in applying pollution prevention strategies (NRMRL)
	3. High Through-Put Screening Endocrine Disruptors-problem-driven research designed to identify endocrine-mediated effects using rapid high through put protein fingerprinting techniques (NHEERL)
C. Enhance Quantitative Risk Assessment	
	1. Dose-Response Assessment Acute-to-Chronic Estimate-problem-driven research to develop regression and accelerated life testing models to predict long-term chronic toxicity from short-term acute responses and determine uncertainty at low concentrations (NHEERL) Develop and Apply Unified Modeling Procedure to Cancer and Noncancer Risk Assessment to support biologically based dose response modeling (NCEA)

	2. Cross Species Extrapolation Cross-species Extrapolation in Birds-problem-driven research to develop PBPK models to extrapolate reproductive and neurological effects of metals among bird species (NHEERL) Value-of-Information Approach to Motivate Uncertainty Factors with Mechanistic Data: Chlorine Human Health Risk Case Study: Experimental and computational efforts aimed at obtaining and integrating mechanism of action data to develop a biologically based risk assessment for chlorine. The results will be generalized to develop a formal framework for departing from default uncertainty factors based on PK/PD data (NCEA)
	3. Chemical Mixtures Chemical Mixtures-core research to apply genomic analyses of exposure of fathead minnows to binary and tertiary chemical mixtures (NERL) Interactions and Mechanism of Pesticide Mixtures: PBPK/BBDR modeling for immunotoxicity risk assessment of chemical mixtures (NCEA)